

A COMPARISON OF THE KINETICS OF ENZYMATIC ADAPTATION IN  
GENETICALLY HOMOGENEOUS AND HETEROGENEOUS  
POPULATIONS OF YEAST<sup>1</sup>

S. SPIEGELMAN

*Lecturer, Washington University*

AND CARL C. LINDEGREN

*Research Professor, Henry Shaw School of Botany of Washington University*

Populations of microorganisms possess the ability of undergoing striking changes in their physiological properties under the stimulation of substances in their environment. Such changes may involve either the acquisition of an enzyme system not previously detectable or the loss of one that it had possessed before the environmental change. An example of this phenomenon is the ability of certain yeasts to acquire the enzymatic apparatus necessary to ferment galactose. Since its discovery by Dienert ('00) this particular problem has been investigated by numerous workers. Armstrong ('05) confirmed Dienert's findings and further found that some yeasts were incapable of acquiring this physiological property no matter how long they were cultured in the presence of galactose. Slator ('08) showed that those yeasts capable of fermenting galactose possess this ability only after they had been acclimatized by culture in its presence. No yeast he investigated was able to ferment this hexose immediately upon being introduced to a medium containing it. There was always an induction period of variable length connected with the acquisition of this property.

Attempts to elucidate further the nature of this acclimatization or adaptation encountered a basic problem common to all studies of physiological changes in large populations. A comparative biochemical study of large populations always involves over-all populational characteristics. This necessarily introduces difficulties in the interpretations of any observed changes in physiological properties. The mechanisms available to an individual cell for adapting itself to an environmental change are limited by its genome and the physiological flexibility permitted by its particular degree of specialization. When, however, the adaptive ability of a population of cells is being considered, there must be added to the physiological pliability of its members the genetic plasticity of the group in terms of the numbers and kinds of variants it is capable of producing.

Because of this composite nature of populational adaptability, it is clear that in any particular case the same end result can be obtained by any one of the following mechanisms: (1) the natural selection of existent variants with the desired characteristics from a heterogeneous population; (2) induction of a new (as far as measurements of activity are concerned) enzyme by the substrate in all the members of a homogeneous population, resulting in an increase in the

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measured enzymatic activity of the population; (3) a combination of natural selection and the action of mechanism (2) on those selected.

Several attempts have been made to decide which of the above mechanisms is involved in the adaptation to galactose fermentation. Sohngen and Coolhaas ('24) grew their yeast cultures at 30° C. and measured enzymatic activity at 38° C. to avoid cell division during the measurement of CO<sub>2</sub> evolution. They concluded from their experiments that the production of galactozymase parallels the formation of new cells. In addition, they confirmed Kluyver's ('14) findings that at 38° C., at which temperature cell division is completely inhibited, no adaptation takes place. Other investigators also tried to obtain adaptation in the absence of cell division since this would clearly exclude the operation of natural selection as a causal agent in effecting the change. Euler and Nilsson ('25), and later Euler and Jansson ('27) in a more thorough examination, tried adapting yeast to galactose fermentation in the presence of 0.5 per cent phenol to inhibit cell division. These attempts failed. They also repeated Kluyver's attempt to dissociate growth and adaptation by culturing the yeasts at 38° C., but again neither growth nor adaptation occurred. Stephenson and Gale ('37), using *Bact. Coli*, also concluded that adaptation to galactose in this organism was invariably accompanied by cell division. No evidence of adaptation was found in the absence of cell multiplication, and the increase in galactozymase activity in the growing population could be explained on the basis of the new cells formed.

The failure of the above-mentioned authors to find adaptation in the complete absence of cell division cannot be taken as conclusive evidence that no such phenomenon could exist. It is conceivable that in cultures where this "ideal" had been reached, the physiological state of the cells was such that their ability to synthesize new enzymes had been lost along with their ability to divide. Stephenson and Yudkin ('36) concluded from their experiments that the production of galactozymase in yeast cultures need not involve the formation of new cells. This conclusion was based on the observation that the ability to evolve CO<sub>2</sub> anaerobically from a medium containing galactose was acquired in a period when the total viable counts of the population remained constant. These adaptive periods were relatively long, extending to 24 hours, and the constancy of count was apparently obtained fortuitously in some of their experiments, though this was aided by using old cultures which had exhausted their polysaccharide reserves.

In view of the apparent contradiction between the findings of Stephenson and Yudkin and those of previous investigators (particularly Sohngen and Coolhaas) the present authors reinvestigated the problem from a different point of view (see Spiegelman, Lindegren and Hedgecock, '44, *hereafter referred to as 1*). They described a method (1) by which the phenotypic homogeneity of yeast populations could be examined with respect to the ability of the individual members to acquire the power to produce CO<sub>2</sub> rapidly from galactose. The method depends on the fact that a cell can generally be characterized by the type of colony it produces when grown on an agar surface under standard conditions.

Thus, if a representative sample of a population is plated out and more than one colonial type is noted, it may be concluded that more than one type of individual is represented in the population (see Shapiro, Spiegelman and Koster, '37). It will be noted that this method of plating out possesses the inherent advantage of providing in the process for the ecological isolation of the individual members of the sample population. This permits a relatively complete expression of any phenotypic heterogeneity which may exist but which might not otherwise be detectable due to the inhibitive competitive interaction amongst the phenotypes.

In order to distinguish between the rapid gas producers (fermenters) and those that could not ferment the sugar, advantage was taken of the fact that colonies which evolve  $\text{CO}_2$  rapidly would, when growing between two layers of agar, produce typical star-shaped cracks in the agar of their immediate neighborhood. The quantitative validity and reproducibility of this detection method were examined and found to be satisfactory.

The reasons for developing this method are evident from the above discussion of the nature of populational adaptability, since one of the crucial points at issue in problems of this nature is the phenotypic homogeneity or heterogeneity of the starting population. Thus, if it can be shown that the initial population was phenotypically heterogeneous with respect to the ability of the individuals to demonstrate a property, then natural selection could operate to produce the change. If, on the other hand, the population can be shown to be homogeneous with respect to this property, any sudden change in the characteristic studied can more likely be ascribed to a direct cytoplasmic interaction with the substrate, the delay being due to an induction period.

Two strains of *Saccharomyces cerevisiae*, Db23B and LK2G12, both of which could acquire the ability to ferment galactose when grown in its presence, were examined by this method. Strain Db23B, which was known to be haploid and therefore genetically unstable (see Lindegren and Lindegren, '43 a, b, c, d, for further details concerning yeast breeding and strain isolation), was shown to be phenotypically heterogeneous with respect to galactose fermentation. It contained two types as far as behavior towards galactose was concerned: One could not adapt to galactose fermentation while the other one could. Strain LK2G12, on the other hand, which was known to be diploid, was uniformly homogeneous in that all of its individuals were able to acquire the capacity for fermentative utilization of galactose on standing in contact with the sugar. The pre-adaptive period of this strain under the conditions of the experiments was three hours, which is characteristic for this particular strain under standard conditions, and has been duplicated many times over a 7-month period. This is apparently much shorter than the periods encountered by Stephenson and Yudkin. Because of the relative rapidity of adaptation of this strain, experiments may be performed on the mechanisms of the process that can rule out cell divisions without drastic inhibitory treatments which might interfere with other physiological functions.

The adaptive behavior of these two strains followed what would be expected from the data obtained on their phenotypic characteristics. Populations of Db23B, starting with a low percentage of the fermenting type, could increase their enzymatic activity only through the mechanism of cell division and the subsequent selection in favor of galactose fermenters. In no case was increased enzymatic activity observed without parallel increase in cell numbers. However, this was not true of LK2G12, in which adaptation could occur without any measurable change in total cell number. From the results with these two strains the existence of both the "natural selection mechanism," as well as that of direct cytoplasmic interaction, was concluded. Which one was operative depended upon the genetic stability and composition of the population.

It will be noted that the conclusion that natural selection operated in changing the characteristics of Db23B populations depended on two types of evidence, namely: the phenotypic heterogeneity of the initial populations with respect to galactose fermentation; and the inability of this strain to increase its enzymatic activity in the absence of cell division when placed in contact with galactose.

As pointed out previously, the absence of increasing enzyme activity in a non-dividing culture has little crucial interpretive value. It does, however, become more meaningful when combined with evidence of phenotypic heterogeneity. Nevertheless, it would clearly be desirable to demonstrate the operation of natural selection more positively by showing its existence in growing populations of Db23B rather than inferring its existence by the absence of adaptation when it is not operating. It is the particular purpose of the present paper to offer such data on growing cultures of Db23B. A theory capable of handling the quantitative analysis of its significance will also be presented. For purposes of comparison similar data will also be presented on two diploid strains, LK2G12 and 812, both of which can adapt without cell division. It will be shown that the time-variation of the ratio of the two phenotypes in a Db23B population growing on galactose is quantitatively described by a "natural selection" mechanism. It will furthermore be shown that the dependence of enzymatic activity on increasing cell numbers in growing populations of Db23B also leads to the conclusion that natural selection is involved.

#### MATERIALS AND METHODS

A. *Yeast strains.*—Three strains of *Saccharomyces cerevisiae* isolated in this laboratory and known as Db23B, LK2G12 and 812 were used in the following experiments. Strain Db23B originated from a single ascospore, and since its population contains principally haploid cells it is characteristically unstable. Strain LK2G12 originated from an intact 4-spored ascus in which copulation was observed to occur pairwise. Consequently it is known to be a diploid and in contrast to the Db23B is characteristically stable. Strain 812 is known to be diploid on similar grounds.

B. *Media.*—The basic medium was made as follows: Into 1 liter of fluid was

dissolved 5 gms. peptone, 1 gm.  $MgSO_4$ , 2 gms.  $KH_2PO_4$ , 4 cc. of 80 per cent sodium lactate, 2 cc. liquid yeast extract, and to this was added the carbohydrate in an amount sufficient to make an 8 per cent solution. Agar plates were made by adding the requisite amount of agar to the above basic medium. The broth medium was always cleared by filtration before distribution to separate flasks for autoclaving. This clearing process was essential in the agar medium to facilitate observation of the colonies on test plates.

*C. Carbohydrates.*—Reagent grade glucose was used. The galactose was Difco's purified further treated according to a method described by Stephenson and Yudkin ('36) to remove any contaminating fermentable sugars.

*D. Test plates.*—These are double-layered agar plates containing 4 per cent galactose purified as described above. Colonies grew between the layers and those that could ferment galactose evolved gas at a sufficient rate to produce the typical star-shaped cracks in the agar in their immediate neighborhood. To test the type composition of a sample population the following procedure was used: The sample was centrifuged down and washed free of any glucose with  $M/15 KH_2PO_4$ , if it came from a glucose medium. The washed cells were then resuspended in chilled galactose broth and diluted to contain approximately 5,000 cells per cc. Of this suspension approximately 0.1 cc. was then placed on a 4 per cent galactose-4 per cent agar surface from which all excess fluid had been allowed to drain. Even distribution was obtained by rotating a sterile bent rod over the surface. A 5 per cent agar medium containing 4 per cent galactose, cooled to  $39^\circ C.$ , was poured over the inoculated surface. These test plates were incubated at  $28^\circ C.$  for at least 48 hours before a count was taken. Usually five test plates were prepared from each suspension it was desired to examine, and the results averaged. Counting was done under the low power of a dissecting microscope.

*E. Manometric measurements.*—Warburg vessels, capable of being flushed with gas, were used. All measurements were taken at  $30.2^\circ C.$ , the vessels being shaken at a rate of 100 oscillations per minute. The nitrogen used to displace the air in measurements of anaerobic  $CO_2$  production was passed over hot copper to remove any traces of oxygen.

*F. Cell counts.*—Cell counts were made by means of a Spencer bright-line haemocytometer.

#### THE "NATURAL SELECTION" HYPOTHESIS

One type of data that is relatively easy to obtain by the use of the galactose test plates is the time-variation of the ratio of fermenters to non-fermenters in a population growing on a galactose medium. It should be possible to use these data to test whether the kinetics of the shift of this ratio in favor of the fermenting type in a growing culture fits that which would be predicted on the basis of a natural selection mechanism. To make such an examination it is necessary to find the functional dependence between the ratio of the two types and time, deducible from a simple selection theory. It would be desirable to transform this

into a linear relation for convenience in testing the goodness of fit.

A functional relation of this type may be found by a simple mathematical description of a selection theory. In the previous paper (1) it was shown that both phenotypes were always present even in glucose-grown cultures. It may be assumed that the fermenters arose through variations from the non-fermenters due to the genetic instability of the haploid populations. This genetic instability is also shown through the numerous morphological variants which such cultures invariably exhibit on plating (Lindegren and Lindegren, '43a).

From the point of view of natural selection the course of the phenomenon subsequent to transferring a glucose-grown Db23B population to a medium containing galactose as the sole source of carbohydrate may be described as follows: The non-fermenters can only use galactose slowly through a purely aerobic type of oxidation<sup>1</sup> and therefore their rate of division is depressed in this medium. The few fermenters present, after a lag period, start to divide rapidly since they possess the enzymatic apparatus necessary to use this sugar at a rapid rate. The number of fermenters thus increases due to two sources: first, the rapid cell division of those already present, and second, the mutation of the non-fermenting type to the fermenting kind. This latter mechanism can, even with relatively low mutation rates, be numerically significant in the early history of the populations because of the relatively large number of non-fermenters initially present. On the other hand, the number of non-fermenters present at any time can increase only by virtue of the cell divisions of this type.

If we let  $U$  represent the number of non-fermenters (unadapted cells) at any time ( $t$ ), and  $A$  the number of fermenters (adapted cells) the above discussion leads to the following two differential equations

$$(1) \quad \frac{dU}{dt} = cU$$

$$(2) \quad \frac{dA}{dt} = aA + bU$$

In these equations  $c$  is the growth constant or "biotic potential" of  $U$  in a galactose medium and  $a$  has a similar significance for the adapted phenotype. The constant  $b$  measures the relative rate at which the  $A$ -type is thrown off during the cell divisions of the  $U$ -type. Equations (1) and (2) describe the growth rate of the two phenotypes in a heterogeneous population. Equation (1) may be solved directly to yield  $U$  as the following function of time,

$$(3) \quad U = U_0 e^{ct}$$

where  $U_0$  is the number of unadapted cells present at zero time. From equation (3) it is seen that

$$c = \frac{1}{t} \ln \frac{U}{U_0}$$

<sup>1</sup> This purely aerobic utilization of galactose by unadapted cells will be described in detail and discussed in a later publication.

and consequently the constant may be obtained as the slope of the line obtained by plotting  $\ln \frac{U}{U_0}$  against time. With the aid of equation (3), equation (2) may be solved to yield

$$(4) \quad A = \left( A_0 - \frac{b U_0}{c-a} \right) e^{at} + \frac{b}{c-a} U_0 e^{ct}$$

where  $A_0$  is the number of adapted cells present at  $t$  equal zero. The magnitude of the constant  $a$  may be obtained from the growth rates of a fully adapted culture when growing on galactose, since under those conditions,

$$\frac{dA}{dt} = aA$$

Equations (3) and (4) express the absolute numbers of both phenotypes as functions of time. Absolute numbers are difficult to obtain with any precision. Furthermore, the use of these equations as they stand to test the theory would involve the relatively accurate determinations of the constants  $a$ ,  $b$  and  $c$ . A simpler way of testing the theory exists in terms of ratios of phenotypes. It will be remembered that the test plates yield data on the ratio of  $A$  to  $U$ , and this  $\frac{A}{U}$  may be obtained directly from equations (3) and (4). To facilitate computations, equation (4) may be simplified without destroying its usefulness in testing the validity of the selection theory. By using the methods described above, the constant  $a$  was found to have a value of 2.1, whereas  $c$  under the same conditions was only 0.1. The constant  $c$  may therefore be neglected in comparison with  $a$  in equation (4) since they both appear always additively. On the same numerical basis  $A_0$  may also be ignored.

These simplifications are ones that do not involve the linearity of the final relation to be tested while contributing to the ease of the mathematical manipulation. Equation (4) thus becomes

$$(5) \quad A = \frac{b}{a} U_0 e^{at} - \frac{b}{a} U_0 e^{ct}$$

Dividing the equation (5) by (3) and again neglecting  $c$  in comparison with  $a$ , we find

$$(6) \quad \frac{A}{U} = \frac{b}{a} (e^{at} - 1)$$

Equation (6) may be put into a more convenient linear form by dividing by  $\frac{b}{a}$ , transposing and taking logarithms of both sides. We thus obtain

$$(7) \quad at = \ln \left[ \left( \frac{a}{b} \right) \frac{A}{U} + 1 \right]$$

The theory then predicts a linear relation between time and the function of  $\frac{A}{U}$

represented on the right-hand side of equation (7). It must be pointed out that the linearity of this relation is independent of the accuracy of the values obtained for the constants  $a$  and  $b$  since they remain the same throughout the experiment. In point of fact, as far as testing the theory is concerned, any arbitrary number may be substituted for the ratio  $\frac{a}{b}$ . The only requirements imposed on these numbers is that  $a$  be assumed finite and  $b$  taken as greater than zero. For purposes of calculations a value of 200 was taken for  $b$ , since several estimates of  $b$  gave values lying between 0.005 to .01.

## EXPERIMENTAL RESULTS

The following typical experiment was performed to test the validity of relation (7) in describing the time-variation of  $\frac{A}{U}$  for a Db23B culture growing on a galactose medium. A two-day-old culture growing in the normal glucose broth medium was centrifuged down and washed twice with M/15  $\text{KH}_2\text{PO}_4$  under sterile conditions. The washed cells were then inoculated into the basic broth medium containing 8 per cent purified galactose as the sole carbohydrate source. The flask containing the culture was then suspended in a water bath at  $30.2^\circ \text{C}$ . and shaken continuously during the experiment. Samples were withdrawn at intervals and diluted with chilled galactose broth to contain approximately 5,000 cells per cc. This diluted suspension was used to prepare 5 galactose-agar test plates, approximately 0.1 cc. being used for each plate. After a suitable incubation period the ratio  $\frac{A}{U}$  was determined from these test plates and the average value taken. The results are tabulated in table I in which are also calculated the values of the function of  $\frac{A}{U}$  to be tested. Figure 1 represents the plot of  $\log \left[ \left( \frac{a}{b} \right) \frac{A}{U} + 1 \right]$  against time. It is clear from the figure that the data do satisfy the linear relation required by the natural selection hypothesis.

TABLE I

DATA EXAMINING THE VARIATION OF A/U WITH TIME IN A GROWING HAPLOID POPULATION TO SEE WHETHER IT SATISFIES THE RELATION PREDICTED BY THE SELECTION THEORY

(A represents the number of fermenters present, U the number of non-fermenters. The ratio  $a/b$  is taken as 200.)

Strain	Hours	A/U	$\log \left[ \left( \frac{a}{b} \right) \frac{A}{U} + 1 \right]$
Db23B	2	0.11	1.301
	4	0.20	1.613
	12	0.49	1.920
	24	4.20	2.700
	32	5.00	3.080
	48	166.00	4.211



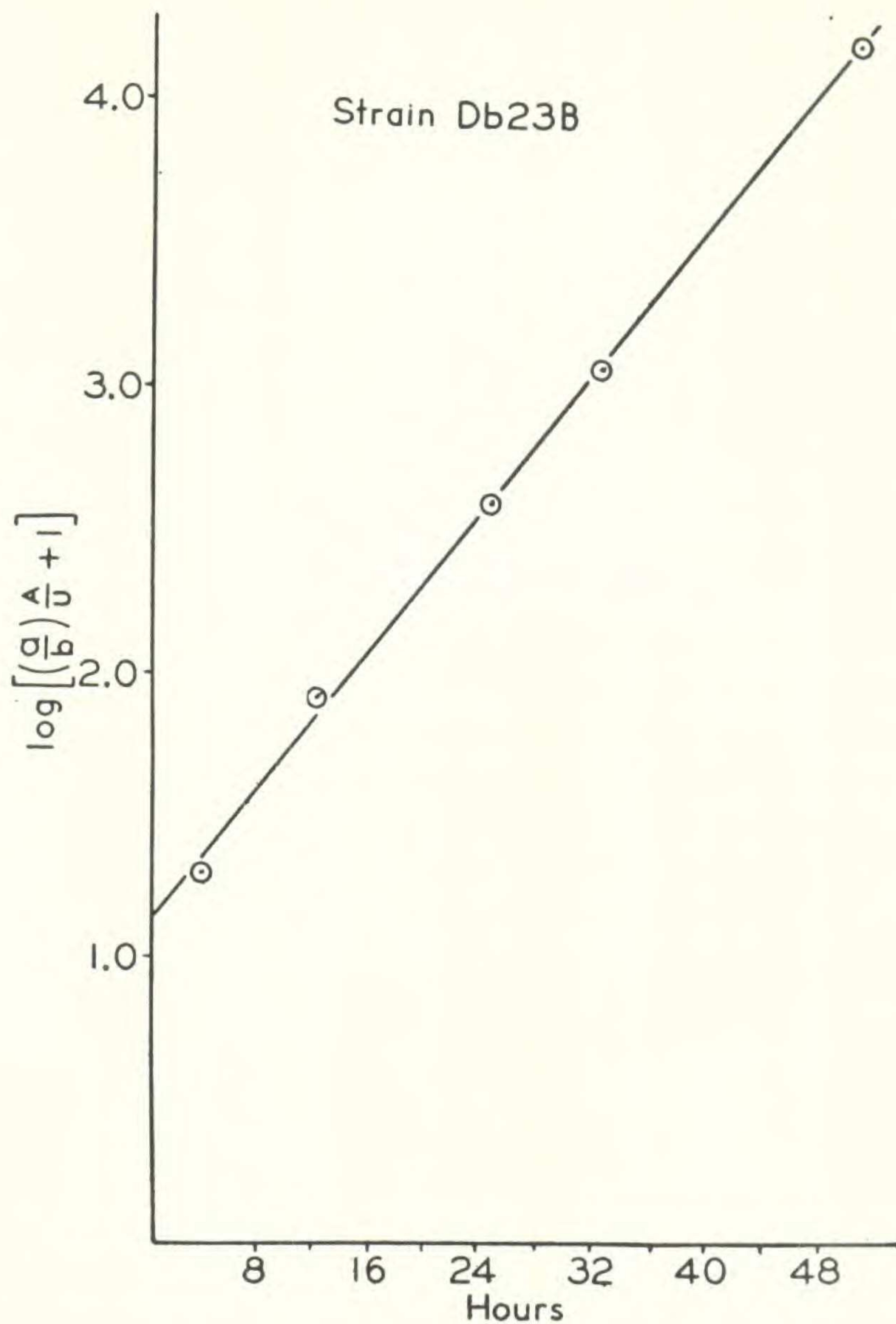


Fig. 1. A plot of data from table 1 to see how closely the time variation of phenotype ratios, obtained with a growing haploid population, satisfies the linear relation predicted by the selection theory.

For comparative purposes an attempt was made to see how well data obtained with strain LK2G12 would fit the theoretical relation deduced from the selection theory. The test plates could not, however, be used to obtain the necessary data, since they measure the ability of acquiring the fermentative enzymatic apparatus, and it has already been shown (see (1)) that populations of this strain are homogeneously positive. The reason for this, of course, exists in the fact that the incubation time (36–48 hours) necessary before the plates can be read by far exceeds the pre-adaptive lag period of the cells of this strain. The following method was used, however, to obtain comparable data. The maximum enzyme activity for this strain under the conditions of the experiment was measured and found to be 165. This number represents the  $Q_{CO_2}^N$  value, i. e., cu. mm. of  $CO_2$

liberated per hour per mg. of dry weight of tissue at normal temperature and pressure. This maximal value of 165 is presumably obtained when all the cells of the population are adapted. If the enzymatic activity of a suspension is less than maximal, it may be assumed, for the purposes of the present discussion, to be due to the fact that unadapted cells form some fraction of the population being tested. Under these conditions we may then take the  $Q_{CO_2}^N$  value measured at any time  $t$  as proportional to  $A$ , the number of adapted cells present, whereas the difference  $(165 - Q_{CO_2}^N)$  may be assumed proportional to  $U$ , the number of unadapted present. Following this reasoning then we may write

$$\frac{A}{U} = \frac{Q_{CO_2}^N}{(165 - Q_{CO_2}^N)}$$

To obtain the necessary data the following experiment was performed: a two-day glucose-grown culture of LK2G12 was centrifuged and washed twice in M/15  $KH_2PO_4$  under sterile conditions. The washed cells were inoculated into basic broth medium containing 8 per cent galactose as the sole source of carbohydrate. The flask containing the culture was shaken continuously during the experiment in a bath at  $30.2^\circ C$ . Samples were removed at intervals and placed in Warburg manometers to determine the rate of  $CO_2$  evolution in an atmosphere of nitrogen. A 20-minute run after equilibration was sufficient to determine the  $Q_{CO_2}^N$  value. Aliquots were taken at the same time for dry-weight determinations. The results of such an experiment, as well as the calculations of the requisite functions from the data, are summarized in table II.

TABLE II  
DATA EXAMINING THE VARIATION OF A/U WITH TIME IN A DIPLOID POPULATION  
(Consult text for method of calculation of A/U.)

Strain	Hours	$Q_{CO_2}^N$	A/U	$\log \left[ \left( \frac{a}{b} \right) \frac{A}{U} + 1 \right]$
LK2G12	1.0	0.01	0.00	0.000
	2.0	0.03	0.00	0.000
	2.8	2.10	0.01	0.401
	3.3	45.00	0.30	1.803

Figure 2 represents a plot of these data similar to the one made for the data obtained with strain Db23B. The theoretical curve, based on the Db23B data, is plotted on the same graph for purposes of comparison since the time scales in the two experiments were necessarily different. It is evident that the kinetics of the appearance of enzymatic activity in a LK2G12 population does not fit a natural selection mechanism.

One other kind of experiment can be performed on a growing culture to check the applicability of the natural selection hypothesis, viz. the type performed by Sohngen and Coolhaas ('24). If this mechanism is operating in producing the change in the enzymatic level, the activity of a culture measured during the

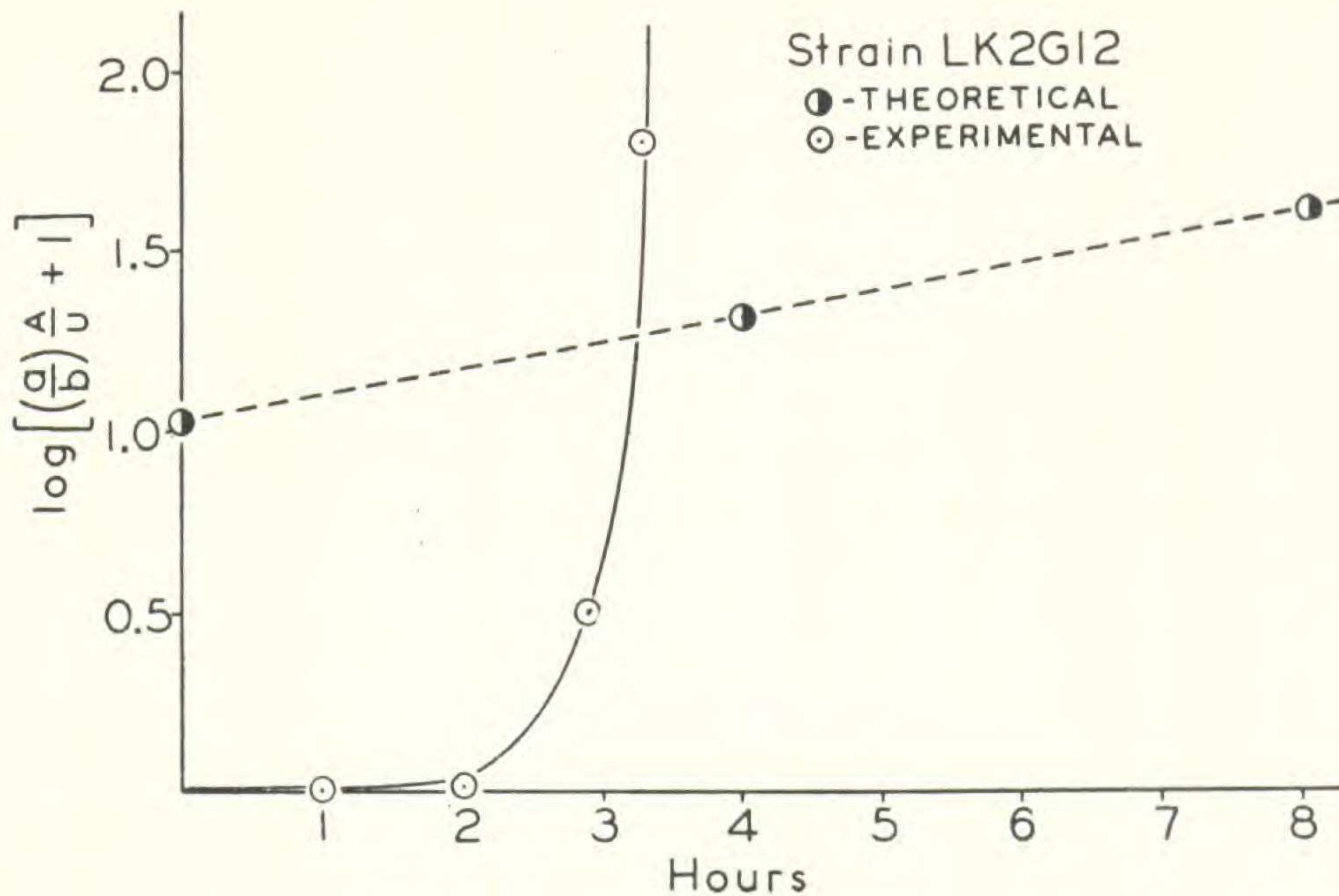


Fig. 2. A plot of data from table II to see how well data on the time variation of phenotype ratios, obtained from a diploid culture, agrees with the linear relation predicted by the selection theory.

growth phase should increase in direct proportion to the number of new cells arising during the period of growth. Thus a plot of enzyme activity against cell number should yield a straight line. It is, however, not necessary to grow the culture at one temperature and test enzyme activity at another as was done by Sohngen and Coolhaas. The Warburg manometric technique is sufficiently sensitive to yield  $Q_{CO_2}^N$  of adequate accuracy by a 15–20-minute run. This period is too short for any marked change to occur in cell count, especially since the cells are under anaerobic conditions. This technique was apparently not available to the above workers, for they used a much cruder aerobic technique.

The following experiment was performed to test whether the increase in enzyme activity observed with growing cultures of Db23B could be accounted for on the basis of the new cells arising during the experimental period. A 2-day glucose-grown culture of Db23B was centrifuged down and washed once in M/15  $KH_2PO_4$ . The washed cells were then resuspended in the basic broth containing 8 per cent of purified galactose as the sole carbohydrate source. The density of the initial suspension was adjusted to contain 15 cells per 16 small squares. This corresponds to 40,000 cells/mm<sup>3</sup>. In practice, the cells were counted in the large squares, each of which was composed of 16 small squares. The data were recorded in terms of number of cells per large square.

The flask was shaken in a bath at 30.2° C. during the experiment. Samples were withdrawn at intervals for cell counts, and measurements were taken of enzyme activity in terms of cu. mm. of  $CO_2$  liberated per 10-minute period per 2 cc. of suspension under anaerobic conditions. Cell counts were always made at the beginning and end of the manometric determinations and in all cases remained the same within the limits of the measurement.

The data obtained with Db23B are summarized in table III. Each value for the rate of CO<sub>2</sub> evolution represents the average over three 10-minute periods or four 5-minute periods. The latter was used in the last three measurements because of increased activity. Each figure in the column giving the number of cells per large square represents the average value obtained by counting 10 such squares in each sample.

TABLE III  
EXAMINATION OF DEPENDENCE OF GALACTOZYMASE ACTIVITY ON INCREASE IN CELL NUMBER IN HAPLOID (Db23B) AND DIPLOID (812) POPULATIONS

Strain	Hours	No. of cells per square	mm. <sup>3</sup> CO <sub>2</sub> evolved per 10 min./2 cc.
Db23B	0	15.7	0.03
	3	18.1	17.1
	4	21.0	16.0
	5	23.8	29.1
	6	26.3	34.0
	7	28.8	47.2
	8	30.1	50.2
812	0	16.6	0.2
	1	15.8	4.0
	2	17.0	23.5
	3	16.9	50.0
	3.5	17.3	70.0

At the same time, for purposes of comparison, a similar experiment was performed with strain 812, which has an adaptation period of two hours and is phenotypically homogeneous. In the case of 812, however, the washed cells were resuspended in M/15 KH<sub>2</sub>PO<sub>4</sub> containing 8 per cent galactose rather than broth.

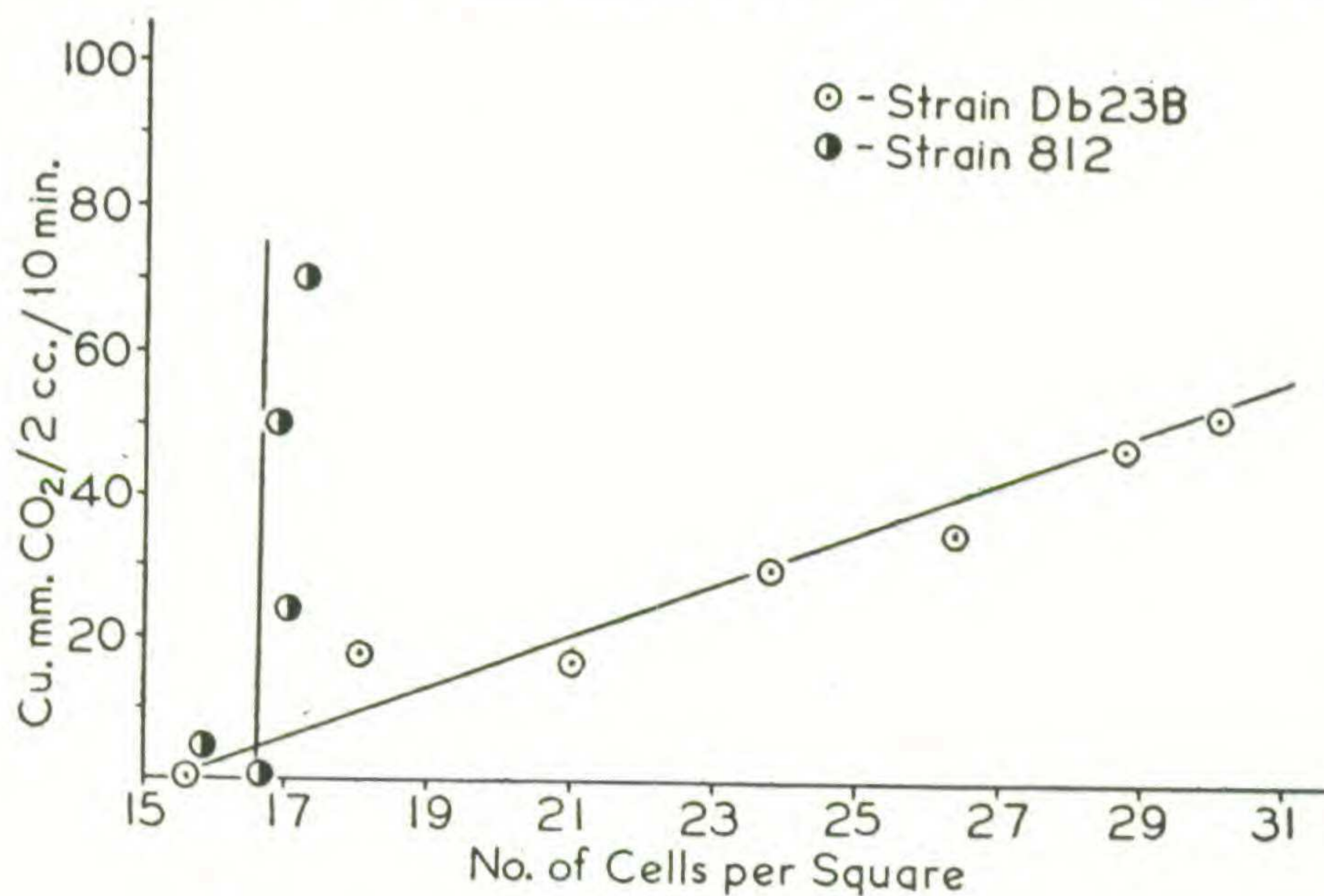


Fig. 3. A comparison of the dependence of galactozymase activity on increase in cell numbers in a haploid (Db23B) and a diploid (812) yeast strain.

This was done to minimize cell division in this experiment and thus accentuate the difference in the mechanisms used by these two strains to attain the change in galactozymase activity. All other experimental conditions were exactly as those imposed in the experiments with Db23B. The data for strain 812 are also recorded in table III. Figure 3 illustrates the type of dependence obtained between cell numbers and increase in enzyme activity. It is strikingly apparent that populations of the haploid strain, Db23B, increase their enzyme activity by virtue of the new cells arising during the experimental period. On the other hand, the measured activity of the 812 population was, in the period examined, virtually independent of cell number. This strain was able to increase its activity from zero to an activity level of 70 while maintaining its population at the same density.

#### DISCUSSION

The examinations of the variation of the two phenotypes with time in growing cultures of the haploid strain, as well as the dependence of enzyme activity on increases in cell number, lead to the conclusion that the appearance and increase of galactozymase activity in cultures of Db23B involve natural selection. Comparison with similar data obtained with the two diploid strains, both of which can adapt without cell division, further strengthens this conclusion. The data presented here on growing populations agree with the results obtained (1) on stationary cultures of the haploid strain.

From these results it is clear that the contradiction noted between the results of Stephenson and Yudkin ('36) and those of earlier workers is only an apparent one and is probably due to the differences in the genetic background and phenotypic constitution of the strains employed.

More generally, the conclusion may be drawn that it is impossible to decide, as some previous authors have tried to do, between the "natural selection" hypothesis and one of "direct cytoplasmic interaction" as the explanation for the production of a particular adaptive enzyme. The existence of one mechanism does not necessarily exclude the other from effecting the production of the same enzyme, as is well illustrated by the nature of the adaptation of galactose fermentation by the haploid and two diploid strains examined.

These experiments then emphasize the important point that the particular biological mechanism involved in the production of a given enzyme or enzyme system in a population of cells is a characteristic of the strain being examined rather than of the enzyme system itself. It is thus meaningless to ask and impossible to answer the question of mechanisms of enzymatic adaptation without referring to the genetic background and stability of the population studied.

The results with Db23B further emphasize the necessity of eliminating natural selection in any experiments the purpose of which is the detailed investigation of the biochemistry of the adaptive process itself. In this strain the rate of increase in measurable enzymatic activity depends not only on the synthesis of the requisite enzymatic systems but also on the rate of cell division. Any procedure which

interferes with the physiology of the latter process necessarily will affect the amount of enzyme measured. For investigations into enzyme synthesis strains should be used which possess (1) a genome which permits the synthesis of the enzyme being studied, and (2) the genetic stability to insure reproducibility of the physiological characteristics of the populations.

The two diploid strains, 812 and LK2G12, both satisfy these requirements and have been used to examine the conditions leading to the synthesis of the galactozymase system and the nature of the preadaptive period. These results will be discussed elsewhere.

#### SUMMARY AND CONCLUSIONS

(1). Data are presented on the kinetics of the replacement of a phenotype incapable of fermenting galactose by one that can acquire the property in genetically unstable haploid populations growing on galactose broth. The nature of the time-variation of the ratio of the two types is shown to fit a relation deduced from a selection theory.

(2). This was compared with comparable data on a diploid strain which did not obey the predicted relation.

(3). The kinetics of the increase in enzyme activity of the haploid populations was studied and shown to depend on the number of new cells which arose. A similar study on a diploid strain showed that increase in enzyme activity could take place in the absence of cell division.

(4). The general significance of these results for the problems of enzymatic synthesis and the induction of new physiological properties in populations of cells is discussed.

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